



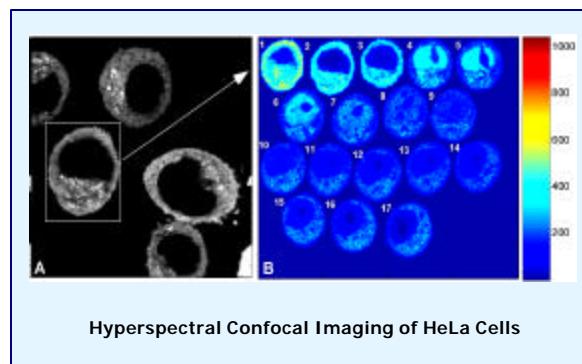
Microscale Immune Studies Laboratory (MISL)

Host-pathogen interactions studied with single-cell resolution

Fact Sheet

Background

Sandia National Laboratories' Microscale Immune Studies Laboratory (MISL) Grand Challenge is a three-year, internally funded (\$3.5 million for FY2006) Laboratory Directed Research & Development Project. Our long-term vision is to develop novel tools to elucidate the molecular mechanisms of the innate immune response at the *single cell level*. The goal of MISL is to understand bacterial and viral pathogenesis by developing an integrated high-throughput experimental and computational approach that provides system-level quantitative, spatio-temporal data at single cell resolution for the TLR4 pathway.

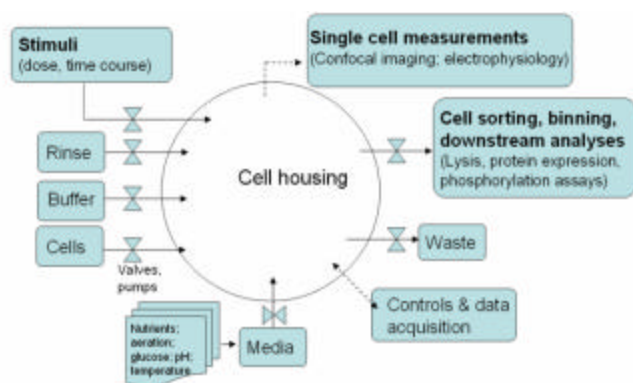


Approach

Towards achieving this goal we have identified four key tasks:

- Creating a suite of fusion proteins and RNAi constructs in host cells to enable precise and quantitative measurement of the response of the TLR4 pathway to pathogenic challenges;
- Developing a versatile high-throughput cellular analysis microfluidic platform for isolating, manipulating, challenging and interrogating single cells and sub-populations of cells;
- Using engineered cells in these systems to make measurements of protein interactions, phosphorylation, localization and expression in host cells; and
- Employing computational models to utilize the experimental data to create a refined dynamic model of TLR4 signaling.

Our integrated microfluidic platform will enable both imaging of single cells and high-throughput acquisition of quantitative protein expression and phosphorylation information of identified cell populations. Two complementary modules are being developed, one centered on a microchip for trapping and imaging viable cells during stimulation and the second for cell sorting followed by protein content and phosphorylation analysis of selected cell sub-populations.



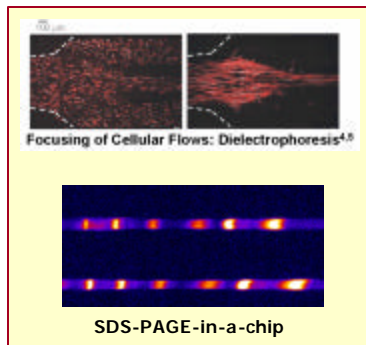
"Life-on-a-Chip" Platform Functionality Requirements

Hyperspectral fluorescence imaging (HSI) with multi-variate curve resolution analysis is used to provide measurements on multiple species simultaneously. Computational modeling is being performed to build dynamic pathway models from the experimental data. Predictive computational models are also being used to generate hypotheses that can guide design of experiments in the integrated platform.

Technical Impact

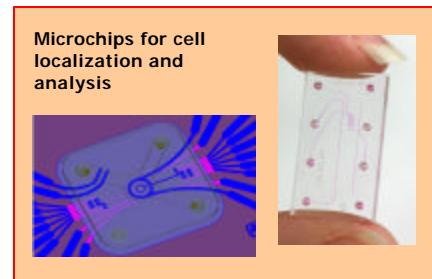
The overarching engineering impact of this project is development of an integrated high-throughput miniaturized platform that combines the functionalities of a confocal microscope, fluorescence-activated cell sorter, and ELISAs.

Recent evidence suggests that very small protein changes in just a few cells may initiate an immune response. Current experimental capabilities cannot measure such changes at the individual cell level in high throughput formats. Our primary technical impact will be to



develop tools that allow single-cell

level experimentation and measurement to elucidate behaviors such as the stochastic nature of immune response, and primary versus secondary signaling by interrogating individual cells and small populations of cells. We will also quantify protein events at a level not currently attainable and have complete control over cell introduction, challenge, and analysis to enable kinetic and quantitative measurements. By combining advances in the biology of host and pathogen cells (GFP fusion constructs, knock-outs and RNAi), microengineered platforms for cell and protein analysis, and predictive computational modeling, our approach will enable a "system-level" understanding of TLR4 pathways.



Benefit to National Needs

While the primary contributions of MISL will be in biodefense and infectious disease research, the fundamental science and device development inherent in the project will directly impact a number of critical areas of national need.

- The microsystems developed will fulfill the fundamental need for compact, high-sensitivity, highly-multiplexed laboratory analytical tools that not only will improve our understanding of interactions between pathogens and hosts but will also aid researchers engaged in drug discovery, cancer research, and clinical diagnostics in a wide range of laboratory environments, including BSL3/4
- In biodefense, our approach will enable us to develop a quantitative spatio-temporal understanding of signaling pathways in innate immunity leading to improvements in early diagnosis of infection by identifying new biomarkers and with the ability to rapidly diagnose the immune response and characterize the threat in terms of its magnitude and effectiveness.
- Troop readiness and efficient field medical diagnosis and treatment are top priorities in national defense. MISL will identify biomolecular markers and provide next-generation device capabilities for battlefield diagnosis and rapid monitoring of the health of the warfighter.

Overall, MISL will revolutionize the way host-pathogen interactions are studied, enabling applications that require high sensitivity detection, rapid and convenient analysis, and comprehensive biological understanding at the systems level.

Major Collaborators

Stanley M. Lemon, M.D., Allan R. Brasier, M.D., and Ashok K. Chopra, Ph.D., University of Texas, Medical Branch, Galveston, TX 77555

William E. Seaman, M.D., UCSF, San Francisco, CA 94121

For More Information, Please Contact

Anup K. Singh, Ph.D., Principal Investigator: (925) 294-1260, aksingh@sandia.gov

Glenn D. Kubiak, Ph.D., Project Manager: (925) 294-3020, kubiak@sandia.gov